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THE PURPLING CHROMOGEN OF A HAWAIIAN DIOSCOREA.

BY

HARLEY HARRIS BARTLETT.

*Chemical Biologist, Drug-Plant, Poisonous-Plant, Physiological,
and Fermentation Investigations.*



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THE PURPLING CHROMOGEN OF A HAWAIIAN DIOSCOREA.

INTRODUCTION.

In 1903 the Office of Foreign Seed and Plant Introduction of the Bureau of Plant Industry received through Mr. Jared G. Smith, Special Agent of the Department of Agriculture in Charge of the Hawaii Agricultural Experiment Station, specimens of a *Dioscorea* under the native name "Hoi."¹ These were inventoried as Nos. 10311 and 10312² and cultivated at the subtropical gardens at Miami and Gotha, Fla. Because of the confused state of the genus it is not now possible to assign a specific name to this *Dioscorea*.³ Perhaps the taxonomy of the group to which it belongs may be treated in a future paper. For the present our plant may be conveniently referred to as the Hawaiian bitter yam. It has recently been offered to the horticultural trade by Childs⁴ under the name of "air potato, or giant yam vine."

The name "air potato" has reference to large aerial tubers which develop in the axils of the leaves. Many species of *Dioscorea* have these tubers, but none exhibits them to greater perfection than the Hawaiian bitter yam. The tubers (shown in natural size in Pl. I) are propagative organs. In this country they are the sole means of reproducing the plant, for all the tubers originally imported seem to have been obtained from pistillate vines. However, the writer is informed by Dr. E. V. Wilcox, Special Agent in Charge of the Hawaii Agricultural Experiment Station at Honolulu, that in Hawaii both pistillate and staminate vines are found. Many yams

¹ The name "Hoi" seems to be of generic application to yams in Polynesia. Hillebrand, commenting on a species which he describes under the name *Dioscorca sativa*, says: "The yam, common in the forests of the lower zone, was cultivated for the supply of ships before the introduction of the potato, particularly on Kauai and Niihau. The species ranges westward over all the regions lying between the Hawaiian Islands and Africa, and its native name, 'Hoi,' follows it to Sumatra. The axillary buds are called 'alaala.'"—Flora of the Hawaiian Islands, p. 438.

² Bulletin 97, Bureau of Plant Industry, entitled "Seeds and Plants Imported during the Period from December, 1903, to December, 1905," p. 9. 1907.

³ In Bulletin 97, Bureau of Plant Industry (loc. cit.), the name *Dioscorea diravicata* was used with a question mark. From this species, however, the plant described in this paper differs in many characters, of which the most striking is the radial rather than dorso-ventral disposition of the tissues of the aerial tuber.

⁴ Childs's Combination Catalogue for 1910, p. 3 of cover.

which, so far as known, have only one sex are propagated exclusively by vegetative means.

There is a chromogen in the aerial tubers of the Hawaiian bitter yam which is of unusual interest in that it appears to be related to the class of ammonia-greening anthocyanins. It has been made the subject of considerable study in the hope that it might afford a clue to the better understanding of these compounds.

OCCURRENCE OF THE CHROMOGEN.

The freshly cut surface of an "air potato" is greenish white, but on exposure to the air it quickly becomes brown by the oxidation of a chromogen (probably a tannin) which has not been investigated. If the freshly cut surface is treated with ammonia, the natural greenish color is greatly intensified, especially in the region of the cambium immediately beneath the cortex.¹ Occasional tubers have pale-purplish instead of greenish flesh, and many greenish tubers become purplish in part when sprouting. If treated with ammonia these purplish tubers also color, just as the greenish ones do, to a somewhat intensified green. On the contrary, treatment with acids will not change the color of a greenish tuber to purplish. If, however, the juice is expressed from a greenish tuber or from the green tips of growing stems and acidified with acetic or hydrochloric acid, a pinkish or purplish color of considerable intensity develops. Nevertheless, the intensity of the green which resulted when a cut tuber was treated with alkali seemed to be out of proportion to the pink which resulted from acidifying the juice. Therefore, in addition to the pigment which was obviously one of the numerous compounds classed as ammonia-greening anthocyanins, a second substance was sought, which like the anthocyanin would give a green reaction with alkali, but unlike the anthocyanin would be colorless or nearly so in acid solution. After much experimenting, such a compound was isolated—a chromogen which in dilute neutral or acid solution is yellowish, which forms intensely wine-red solutions on oxidation with plant oxidases or weak inorganic oxidants and intensely green alkali salts both before and after oxidation.

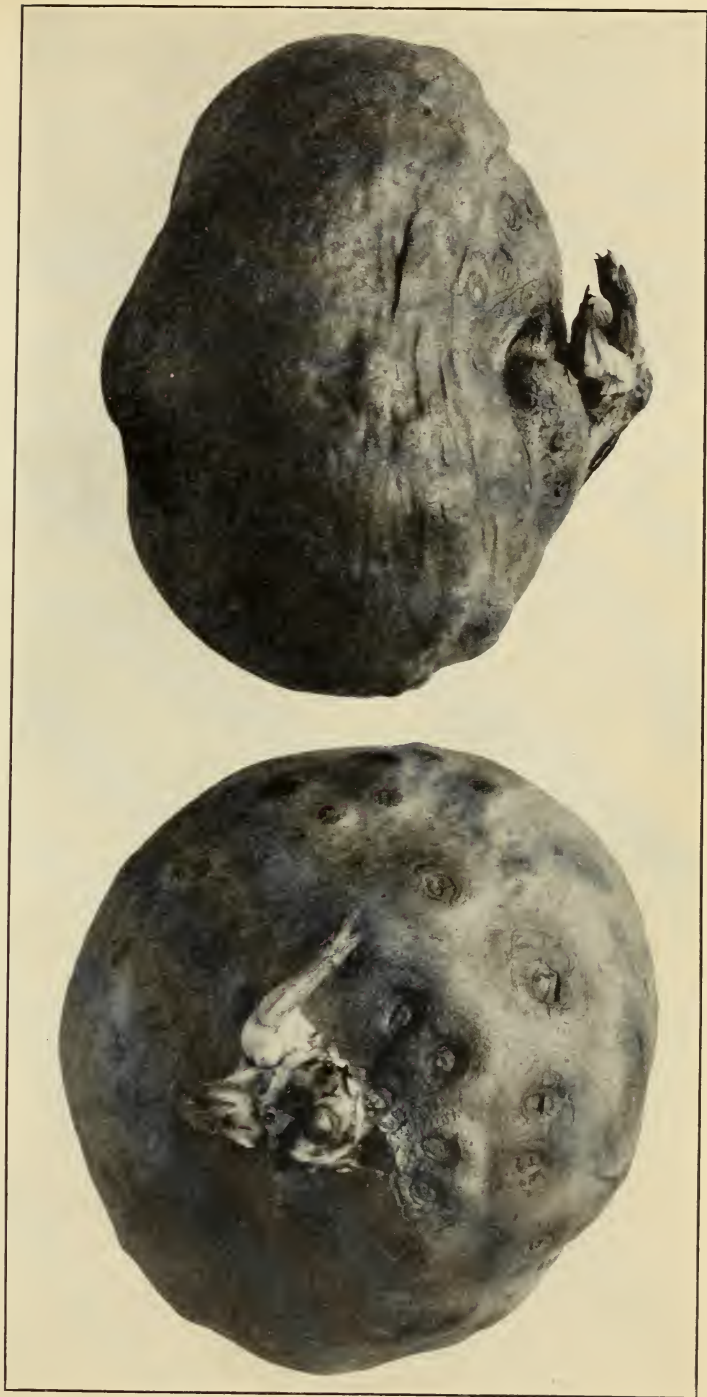
ISOLATION OF THE CHROMOGEN.

As the result of several trials the following method is recommended for the isolation of the chromogen in a comparatively pure state.

¹ In regard to the anomalous morphology of the *Dioscorea* tubers, see the following: Bucherer, Emil. Beiträge zur Morphologie und Anatomie der Dioscoreaceen. Bibliotheca Botanica, no. 16, 1889.

Dale, Elizabeth. On the Origin, Development, and Morphological Nature of the Aerial Tubers in *Dioscorea Sativa*. Annals of Botany, vol. 15, 1901, pp. 491-501.

Goebel, K. Die Knollen der Dioscoreen und die Wurzelträger der Selaginellen, Organe, welche zwischen Wurzeln und Sprossen stehen. Flora, vol. 95, 1905, pp. 167-212.



AERIAL TUBERS OF THE HAWAIIAN BITTER YAM.
(Natural size.)

The tubers are sliced into narrow strips and dried as expeditiously as possible at 60° C., or if plenty of material is available the tubers are pared and the parings only are dried. The dry material after having been ground is extracted at the room temperature, first with ether and then with a mixture of equal volumes of ether and alcohol. To the ether extract a small quantity of alcohol is added and the ether distilled off, leaving the chromogen in alcoholic solution. This is mixed with petroleum ether, and water is added to bring about a separation of a petroleum-ether layer (containing wax, etc.) from the alcoholic layer (containing the chromogen). The latter is added to the ether-alcohol extract.

The ether-alcohol extract is distilled until the residue is free from ether and considerably concentrated. It is then mixed with a cold, concentrated calcium-chlorid solution (or mixed with water and saturated with common salt) and shaken first with several portions of petroleum ether to remove various impurities, and then with acetic ether to remove the chromogen.¹ The chromogen in acetic-ether solution is then shaken with a large excess of concentrated aqueous lead-acetate solution. The copious gummy precipitate, most of which remains suspended in the acetic-ether layer, is filtered off on a Buchner funnel and washed with acetic ether. The acetic-ether filtrate, which is of a clear golden-yellow color, is now shaken successively with solutions of ferrous sulphate (strictly free from ferric salts and dissolved in recently boiled water) and bipotassium hydrogen phosphate and finally several times with distilled water. After filtering the solution it is poured into a large excess of petroleum ether. The chromogen is thrown down as a finely divided, white, opalescent precipitate, which speedily coalesces to form a soft, brown, resinous mass. In this condition the chromogen contains much acetic ether. It may be partially dried in a vacuum desiccator, then pulverized as much as possible, and dried at 78° C. in a vacuum drying oven in a slow stream of rarefied hydrogen.

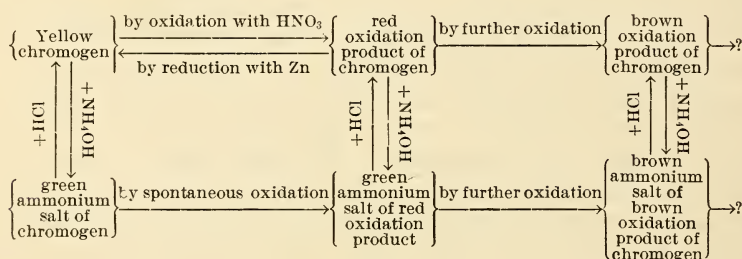
PHYSICAL AND CHEMICAL PROPERTIES.

The chromogen as obtained by the process outlined is a brown resinous compound soluble in alcohol, acetic ether, and chloroform, moderately soluble in ether, and insoluble in petroleum ether and water. Its melting point is not sharp, and it has not been possible

¹ The saline alcohol and water solution which remains after this operation soon changes from yellow, which color is imparted to it by a small residue of unextracted chromogen, to a fine purple. The purple compound is the same oxidation product of the chromogen which is mentioned on page 10 of this bulletin. Its formation in the presence of chlorids is no doubt analogous to the bluing of guaiac resin by chlorids, as described by Alsberg. (See Beiträge zur Kenntnis der Guajak-Reaktion, Schmiedeberg-Festschrift, 1908, Supplement, Archiv für Experimentelle Pathologie und Pharmakologie, pp. 39-53.) The oxidation product is precipitated by lead acetate, so that any small quantity which may contaminate the chromogen after salting out is removed in the next operation.

to obtain it in a crystalline condition. It is fairly stable in neutral solutions, and in alcoholic solution is unchanged by boiling with zinc dust under a reflux condenser. Prolonged heating in contact with the air, more particularly when a reducing substance, such as zinc, is not present, results in its destruction by oxidation. Acid solutions, which are of the same color as neutral solutions, slowly deposit insoluble brown compounds, with simultaneous destruction of the chromogen.

The chromogen is the first member of a series of acids. The other members are successive oxidation products of the chromogen. Each acid forms a series of salts. The relationship of these derivative compounds is as follows:



The oxidation of the chromogen to its red derivative and the formation of the intensely green ammonium salt of either the chromogen or its red derivative constitute the two characteristic color reactions which are mentioned on page 8.

The red oxidation product is best observed when the chromogen is dissolved in acetic ether and shaken with water containing a trace of fuming nitric acid or ferric chlorid. Under these conditions a beautiful wine-red acetic-ether solution of the oxidation product is obtained. If it is carefully washed by repeatedly shaking with distilled water the red solution may be preserved for several days, but not indefinitely, for oxidation will spontaneously proceed until the solution becomes brown by the formation of a further oxidation product. Even this brown compound does not represent the final stage of the oxidation of the chromogen, as a further change is indicated by the ultimate deposition from solution of a substance insoluble in acetic ether. The red stage in the oxidation of the chromogen is very easily overstepped, with the immediate formation of brown compounds, if the mineral oxidants used are too concentrated or if the chromogen is not protected from a too violent oxidation by being dissolved in a solvent, such as acetic ether or chloroform, which is immiscible with the solvent of the oxidant (water).

The chromogen may be oxidized, in aqueous solutions containing sufficient alcohol to prevent precipitation, through the agency of plant oxidases (see p. 12) or of halogen salts (see p. 9). Under such cir-

cumstances a purple solution is obtained. On this account the adjective "purpling" has been applied to the chromogen in the title of this paper. The purple compound which is formed in aqueous solution is identical with the red compound which is formed in acetic-ether solution. It may be completely removed from aqueous solutions by shaking with acetic ether. In water it is purple, in organic solvents red. The red compound is too unstable to be obtained in a solid state by the evaporation of its acetic-ether solution.

When the chromogen or one of its oxidation products is treated with an alkali a salt is formed. In striking contrast to the series of acids, the salts are all totally insoluble in acetic ether or chloroform, but very soluble in water. The salts of both the chromogen and its red oxidation product are deep green in aqueous solution. If a solution of the chromogen or of the red compound in acetic ether is floated on dilute ammonium hydroxid the corresponding ammonium salt is formed at the juncture of the two liquids. After the layers are shaken together the deep-green aqueous salt solution will settle out, leaving the acetic ether colorless. By immediately acidifying the mixture and shaking it the process may be reversed.

The ammonium salt of the chromogen in aqueous solution absorbs oxygen from the air with avidity, passing first, without visible change, to the similar salt of the red oxidation product. If the salt is acidified at this stage the red compound is obtained, and the chromogen itself may be recovered therefrom by reduction with zinc. On longer standing, however, the green salt solution becomes brown. If it is then acidified and shaken with acetic ether a brown acetic-ether solution is obtained, which to all appearances contains the same brown oxidation product of the chromogen that results when the chromogen is directly oxidized beyond the red stage by nitric acid.

Dr. P. G. Nutting, of the Bureau of Standards, has kindly photographed and absorption spectra of the chromogen, the wine-red oxidation product, and the two green alkali salts. From an examination of the negatives he has reported as follows:

The yellow chromogen solution, 1:2,000 in acetic ether, 21 millimeters thick, shows complete absorption of the ultra-violet. Transmission begins gradually at 0.39, just at the limit of the visible violet, and increases steadily to full value in the greenish blue at wave length 0.52.

The red oxidized chromogen, 1:2,000 in acetic ether, 21 millimeters thick, shows complete absorption of violet, blue, and green, gradually increasing transmission through the yellow and orange, and full transmission of the red. Transmission begins at wave length 0.55 in the greenish yellow and becomes full at 0.63 in the red.

The green ammonium salts, 1:4,000 and 1:40,000 in aqueous solution, showed absorption so nearly nonselective that no determinations were made upon them.

When the chromogen is oxidized by plant oxidases it need not be protected from the oxidant by solution in a solvent immiscible with

water. Potato juice (oxidase), prepared by grinding potato parings and pressing through raw silk, actively oxidizes chromogen, which is added in alcoholic solution, first, to the wine-red compound and then to the more highly oxidized brown compound. The oxidation by potato juice is accompanied by measurable oxygen absorption from the air. The potato oxidase is much more active when oxygen is supplied by hydrogen peroxid. Horse-radish juice alone (peroxidase) fails to oxidize, even upon prolonged shaking, as is shown by the fact that the chromogen may be recovered unchanged in color, and by the additional fact that there is no measurable oxygen absorption. The tests for oxygen absorption were conducted with the apparatus designed and described by Bunzel.¹ Hydrogen peroxid alone does not oxidize the chromogen, but in the presence of hydrogen peroxid horse-radish juice is a powerful oxidant, carrying the oxidation almost instantaneously to the wine-red stage, at which it halts. The chromogen is as sensitive in its reactions to oxidases and peroxidases as guaiac resin.

Juice expressed from the *Dioscorea* tubers given rather unsatisfactory results as an oxidase solution because of its highly mucilaginous nature and because of the large quantity of easily oxidized tannin which it contains. Nevertheless, juice pressed from parings by a hydraulic press readily oxidized the chromogen in the presence of hydrogen peroxid to the same wine-red compound which was obtained when other plant juices or inorganic oxidants were used. The presence of hydrogen peroxid seemed to be essential to the reaction; that is, to use the terminology of Chodat, the tuber contains a peroxidase but no associated oxygenase. A moderately active oxidase solution was prepared from green tips of the stems of tubers which had sprouted in the laboratory by mashing with water and filtering. This solution gave (1) no reaction with chromogen alone, (2) an immediate darkening and eventually a deep-purple color with chromogen plus hydrogen peroxid, and (3) a more delayed but eventually pronounced purpling with hydrogen peroxid alone. In the second case, the purple-colored substance was of the usual wine-red color when shaken out into acetic ether. In the third case, the apparent indication that a chromogen was present in the juice led to a further investigation of the stems in the hope of finding in what form, if any, the chromogen of the tubers was translocated to the growing parts.

Several tubers were allowed to sprout in a dark chamber until the white etiolated shoots were 6 inches to a foot in length. These were cut into small pieces, discarding only the purple nodes and rudimentary leaves, and dropped into boiling acetic ether. The material thus

¹ Bunzel, H. H. The Measurement of the Oxidase Content of Plant Juices. Bulletin 238, Bureau of Plant Industry. 1912.

killed was ground in a mortar with the acetic ether and the solution filtered. The resulting pale-yellow solution was shaken with water and the two layers separately examined. The acetic-ether layer contained no chromogen which could be demonstrated by oxidation with dilute fuming nitric acid or by the formation of a characteristic alkali salt. The colorless water layer gave an immediate darkening (green passing quickly to brown) with ammonia, and became rose pink on the addition of acetic acid. The color obtained when the solution was treated with dilute fuming nitric acid was of the same intensity as that obtained with acetic acid, and it was therefore concluded that the substance giving the reactions was the water-soluble anthocyanin of the stems. The rose-pink compound formed by acidifying the solution could not be shaken out into organic solvents. Since the chromogen of the tuber is insoluble in water it would be surprising to find it in the stem. If, as seems likely, the chromogen is concerned with the formation of the water-soluble anthocyanin, the change to a soluble compound takes place in the tuber. In this connection it should be pointed out that no difference could be observed between the water-soluble anthocyanin of the stems and that of the tubers. (See p. 8.) The purpling of stem juice with hydrogen peroxid alone would seem to be due to the slight acidity of commercial solutions of this reagent.

EXISTENCE OF A SECOND CHROMOGEN.

It remains to call attention to a second chromogen which occurs in the tubers. The precipitate obtained when the ammonia-greening chromogen was treated with lead acetate (see p. 9) was dried and triturated with acetic ether slightly acidified with acetic acid. A small part of the precipitate was thereby decomposed. After filtration the filtrate was neutralized by shaking with bipotassium hydrogen phosphate and washed with water. Precipitation with lead acetate and recovery by suspension in acidified acetic ether were repeated, and the chromogen precipitated in a relatively pure condition by adding petroleum ether. It formed a brown resinous mass, similar in appearance to the ammonia-greening chromogen already described. Its yellow solution in acetic ether yielded a water-soluble purple ammonium salt on shaking with dilute ammonium hydroxid. This salt passed entirely into the ammoniacal aqueous layer, leaving the acetic ether colorless. On standing, it quickly oxidized to a brown compound, but if reacidified immediately after its formation the yellow unchanged chromogen could be recovered in the acetic ether. This ammonia-purpling chromogen gives an intensely red compound when oxidized by shaking its acetic-ether solution either with greatly diluted fuming nitric acid or with horse-radish peroxidase + hydro-

gen peroxid. The ammonium salt of the red oxidation product is a purple compound, insoluble in both water and acetic ether.

NOMENCLATURE.

It is proposed to designate the ammonia-greening chromogen of the *Dioscorea* tuber as rhodochlorogen. In so doing, the precedent set by Reinke¹ is followed. This author gave the name rhodogen (formed on the analogy of the word chromogen) to the colorless substance from which on oxidation he obtained the red coloring matter of beets. The word "rhodochlorogen" (from *ῥόδον*, rose, *χλωρός*, green, and *γενής*, producing) is formed with reference to the colors of the oxidation product and the alkali salts. It may be used as a generic designation for chromogens which appear to be related to the ammonia-greening anthocyanins, until the chemistry of these compounds is better understood. The ammonia-purpling chromogen of the *Dioscorea* tuber is hardly well enough established as a chemical individual to warrant applying even a temporary designation to it.

POSSIBLE RELATIONSHIP WITH THE ANTHOCYANINS.

In order to fix the characteristics of the two *Dioscorea* chromogens in mind, they are contrasted briefly in Table I before proceeding with a discussion of their possible relationship to the anthocyanins.

TABLE I.—*Comparison of two Dioscorea chromogens.*

	Rhodochlorogen.	Ammonia-purpling chromogen.
Solution in acetic ether.....	Yellow.....	Yellow.
Ammonium salt formed in acetic ether.....	Insoluble green precipitate....	Purple, partly soluble.
Ammonium salt shaken into water.....	Deep-green solution.....	Purple solution.
Water solution of ammonium salt acidified after partial spontaneous oxidation and shaken with acetic ether.	Purple acetic-ether solution....	Insoluble purple precipitate.
Oxidized by peroxidase + hydrogen peroxid in very dilute alcoholic solution.	Purple solution.....	Red-purple solution.
Oxidized by nitric acid in acetic-ether solution.	Wine-red solution.....	Brownish red solution, eventually a purple precipitate.
Ammonium salt of oxidation product formed in acetic ether.	Insoluble green precipitate....	Insoluble purple precipitate.
Ammonium salt of oxidation product in water.	Deep-green solution.....	Insoluble purple precipitate.

The similarity of these chromogens to the anthocyanins at once suggests itself. There is present in the stems and petioles of the bitter yam a water-soluble cell-sap color which belongs to the

¹ Reinke, J. Ein Beitrag zur Kenntnis leicht oxydirbaren Verbindungen des Pflanzenkörpers. Zeitschrift für Physiologische Chemie, vol. 6, 1882, p. 263. " * * * in der lebenden Pflanzenzelle leicht oxydirbare Substanzen vorhanden sind, welche begierig atmosphärischen Sauerstoff anziehen, und mit demselben Oxydationsprodukte bilden." In referring to the chromogen which produces the red coloring matter of beets, Reinke says, "Weil der Sauerstoff der atmosphärischen Luft diese Substanz zu einem roten Farbstoff zu oxydiren vermag, will ich dieselbe als Rhodogen bezeichnen * * *."

apex of the petioles (see fig. 1). It forms green salts with alkalis, ammonia-greening class of anthocyanins. It is of a very deep purple-red color and is particularly conspicuous in the enlarged base and which on standing spontaneously oxidize to brown compounds. It may be reduced by boiling with zinc dust to a yellow compound, from which the anthocyanin is regenerated by oxidation. (The oxidation of the yellow reduction product takes place spontaneously when its solution is exposed to the air.) This yellow reduction compound of the anthocyanin is strictly comparable to rhodochlorogen in that it forms green alkali salts. Rhodochlorogen oxidizes to a purple or red compound, depending upon the solvent. The yellow reduction product reoxidizes to purple or reddish purple anthocyanin. Thus far, the similarity of the two compounds is perfect. Just as the yellow rhodochlorogen and its purple or red oxidation product both form green salts, so the purple anthocyanin and its yellow reduction compound both form green salts. The two pairs of compounds are strikingly unlike only in their solubility relations, rhodochlorogen and its red oxidation compound being soluble in acetic ether or chloroform but not in water, whereas the anthocyanin and its yellow reduction compound are soluble in water but not in acetic ether or chloroform. The anthocyanin has not been isolated and is therefore not known to be a glucosid, but it seems probable, in view of Grafe's investigations, that the two water-soluble compounds are glucosids of the two which are not water soluble. Even if they are not related in this way, there is strong circumstantial evidence that the chromophoric nucleus in the molecules of rhodochlorogen and anthocyanin is identical and therefore that the two substances are in some way genetically related in the plant metabolism.

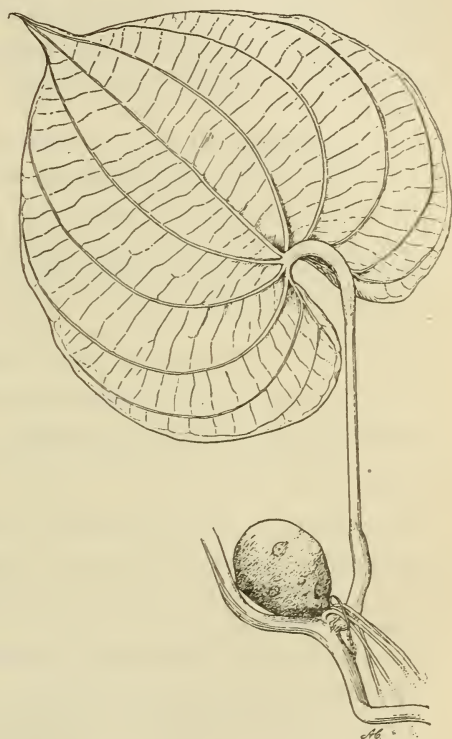


FIG. 1.—Leaf of *Dioscorea* showing petiole enlarged at base and apex, immature aerial tuber, and bases of racemes. (Reduced to one-half diameter.)

In this connection attention should be called to the fact that Grafe¹ has resolved the ammonia-greening anthocyanin of *Althaea rosea* into two components. One, a water-soluble sap color of the usual type, is a glucosid of the formula $C_{20}H_{30}O_{13}$. The other, insoluble in water but soluble in absolute alcohol, is not a glucosid. Its formula $C_{14}H_{16}O_6$ differs from that of the glucosid by a molecule of water and a molecule of glucose. In their color reactions the two components are similar, and there is little doubt that they contain the same chromophoric nucleus. The nonglucosid, which is insoluble in water, greatly resembles the red oxidation product of rhodochlorogen in that it may be reduced to a yellow compound which seems to be analogous to rhodochlorogen itself.²

Glan³ had already shown before Grafe took up his work on the mallow pigments that the water-soluble anthocyanin of *Althaea rosea* was capable of reduction to a colorless compound (yellow if in sufficiently concentrated solution?) which again spontaneously reverted to the anthocyanin by oxygen absorption. In this respect it is similar to the Dioscorea anthocyanin. There seems to be a general parallelism between Grafe's compounds and those which occur in the Hawaiian yam. Not too much stress can be laid upon this parallelism, however, in view of the fact that rhodochlorogen has not been adequately studied either from a chemical or from a physiological standpoint, and its genetic relationship to a water-soluble anthocyanin, although in the highest degree probable, remains unproved.

In regard to the ammonia-purpling chromogen of Dioscorea tubers little can be said. It has been obtained in sufficient quantity for only a few tests and was probably not even approximately pure. It may be pointed out, however, that in its color reactions it resembles some of the nonammonia-greening anthocyanins.

¹ Grafe, Viktor. Studien über das Anthokyan.—I. Sitzungsberichte der mathematisch-naturwissenschaftlichen Klasse der kaiserlichen Akademie der Wissenschaften, vol. 115, pt. 1, 1906, p. 975; II, vol. 118, pt. 1, 1909, p. 1033. Since this paper was submitted for publication (June 24, 1912) the third article of this series has reached the writer.

² "Bei der Reduktion mit Jodwasserstoffsäure entsteht eine gelbe Substanz, die durch nachfolgendes Schmelzen mit Aetzkali Protocatechusäure oder Brenzkatechin ergibt. Der Zusammenhang des Malvenanthokyans mit Gerbstoffen oder Substanzen der Xanthon-Flavongruppe wird dadurch und mit Rücksicht auf die Versuche anderer wahrscheinlich gemacht."—Grafe, Viktor, op. cit., II, vol. 118, pt. 1, 1909 p. 1044.

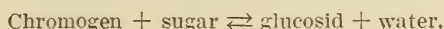
³ Glan, Rudolf. Ueber den Farbstoff der schwarzen Malve (*Althaea rosea*). Inaugural Dissertation, Erlangen, 1892. "Der Farbstoff der Malvenblüten gibt mit Säuren eine rote, bei der Neutralization mit Basen eine blaue und mit überschüssigen Basen eine grüne Färbung * * * Reduktionsmittel entfärben denselben, doch kann er durch Sauerstoffaufnahme noch regeneriert werden * * *. Die Einwirkung von verdünnter Schwefelsäure auf den Farbstoff der Malve liefert Dextrose und ein Spaltungsprodukt, welches Acetylgruppen aufzunehmen vermag, mit Alkalien Brenzkatechin, zuletzt reichlich Protocatechusäure liefert. Der Farbstoff hat Glycosidcharakter und darf als mit Dextrose combinirtes Protocatechusäurederivat aufgefasst werden."

WHELDALE'S THEORY OF ANTHOCYANIN FORMATION.

Miss Wheldale¹ has published during the last four years several papers on anthocyanin and flower pigmentation which have attracted wide attention. Since the work on the chromogens and anthocyanin of *Dioscorea* relates directly to the subject upon which she has been working, it will not be amiss to point out its bearing upon her views. In an article in the *Journal of Genetics* (1911) she has summarized her theory of anthocyanin formation in a series of propositions of which the following now concern us.

(1) The soluble pigments of flowering plants, collectively termed anthocyanin, are oxidation products of colorless chromogens of an aromatic nature which are present in the living tissues in combination with sugar as glucosids.

(2) The process of formation of the glucosid from chromogen and sugar is of the nature of a reversible enzyme action:



(3) The chromogen can be oxidized to anthocyanin only after liberation from the glucosid, and the process of oxidation is carried out by one or more oxidizing enzymes:



(4) From Nos. 2 and 3 we may deduce that the amount of free chromogen, and hence the quantity of pigment formed at any time in a tissue, is inversely proportional to the concentration of sugar and directly proportional to the concentration of glucosid in that tissue.

Inasmuch as the one water-soluble ammonia-greening anthocyanin of which we have reliable chemical information is itself a glucosid, it is obvious that Miss Wheldale's conclusion that water-soluble anthocyanins are formed by the oxidation of nonglucosidal chromogens is of dubious value as a general proposition. It becomes still more dubious when we consider that the only nonglucosidal ammonia-greening anthocyanin known (that of *Althaea*) is insoluble in water and that the only nonglucosidal chromogen which on oxidation gives a compound resembling an ammonia-greening anthocyanin (the rhodochlorogen of *Dioscorea*) yields oxidation products which do not dissolve in water. Furthermore, the regeneration of water-soluble anthocyanins from their yellow reduction products takes

¹ Wheldale, M. *The Colours and Pigments of Flowers, with Special Reference to Genetics*. Proceedings, Royal Society of London, ser. B, 1909, pp. 44-60.

——— On the Nature of Anthocyanin. Proceedings, Cambridge Philosophical Society, vol. 15, 1909, p. 137.

——— Plant Oxidases and the Chemical Interrelationships of Colour-Varieties. *Progressus Rei Botanicae*, vol. 3, 1910, pp. 457-473.

——— On the Formation of Anthocyanin. *Journal of Genetics*, vol. 1, 1911, pp. 133-158. Review by Molisch, *Zeitschrift für Botanik*, vol. 4, pp. 135-136. Review by Hagem, *ibid.*, pp. 136-138. 1912.

place spontaneously in the presence of oxygen and does not require the agency of oxidizing enzymes. This is true not only of the water-soluble anthocyanins of *Althaea* and *Dioscorea*, but also of the similar ammonia-greening anthocyanin of red cabbage, the ordinary reactions of which are familiar to many chemists on account of its occasional use as an indicator. Of course, it is not impossible that the chromogen which is oxidized to anthocyanin in the plant cell is different from the artificially obtained reduction product of the same anthocyanin. The fact may be emphasized at this point, however, that rhodochlorogen gave an identical product with plant oxidases and with mineral oxidizing agents.

It is clear that too little is known regarding the physiological significance and chemistry of anthocyanins, which are chemically very diverse compounds, to warrant anyone in speculating on the mode of their formation in the plant cell. Miss Wheldale's speculations are too obviously without adequate experimental basis.

CONCLUSION.

A chromogen isolated from the aerial tubers of the Hawaiian bitter yam has been named "rhodochlorogen." It has possible chemical and physiological relationships with the ammonia-greening anthocyanin of that plant in that it forms green salts and oxidizes to a red compound which would itself pass from an anthocyanin if it were not insoluble in water. Many investigators have supposed that the anthocyanins were closely allied to the tannins. In this connection it is especially interesting that during the process of purification rhodochlorogen was separated from tannin by the use of lead acetate and ferrous sulphate. Lead acetate did not precipitate rhodochlorogen, but did precipitate its red oxidation derivative.

There is reason to believe that rhodochlorogen of *Dioscorea* contains the same chromophoric nucleus as the anthocyanin of the same plant; consequently, the reactions of rhodochlorogen afford evidence against Miss Wheldale's hypothesis that the so-called ammonia-greening anthocyanins are not autonomous compounds but merely mixtures of yellow flavones with ammonia-bluening anthocyanins, mixtures which would give green as a resultant color with alkalis.¹ Grafe² has already concluded that the ammonia-greening reaction

¹ "The pigment (anthocyanin) closely resembles the flavone in its general behavior toward chemical reagents except that the range of color reactions is different. The oxidized product gives a blue color with alkalis, whereas the chromogen, as previously stated, gives a yellow color. The result, therefore, of alkali action on the combination is green, due to the mixture of yellow and blue."—Wheldale, *Progressus Rei Botanicae*, vol. 3, 1910, p. 468.

² Die grüne Reaktion des Anthokyans, welche mit einem Überschuss an Alkali eintritt, dürfte entgegen der älteren Anschauung nicht oder wenigstens nicht in allen Anthokyanen auf die Anwesenheit von Gerbstoffen zurückzuführen, sondern eine dem Anthokyan selbst eigentümliche Eigenschaft sein."—Grafe, Viktor, op. cit., I, vol. 115, pt. 1, 1906, p. 993.

of certain anthocyanins is not due to the admixture of tannins. This conclusion is confirmed by the work on the rhodochlorogen of *Dioscorea*.

The only yellow compounds known which oxidize to anthocyanin do so spontaneously, and the reaction does not involve the agency of oxidases. Although rhodochlorogen is easily oxidized by plant oxidases to its red oxidation derivative, there is as yet no evidence whatever that this process takes place in the plant cell. It seems more likely that rhodochlorogen is transformed first into a glucosid and that this oxidizes to anthocyanin than that the chromogen is directly oxidized in the plant cell. As yet, however, it is not known that rhodochlorogen has any further part in metabolism after it is laid down in the tuber. It may be nothing more than an end product.

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